page 2, line 24 through page 4, line 4, and at page 6, lines 3 through 23. The Abstract has also been amended to remove the term "pROTECT." No new matter is added by these amendments.

Applicant submits that the subject matter recited in new Claims 6-15 corresponds to the subject matter recited in original Claims 1-3 and 5. For example, original Claim 1 and new Claims 6-11 feature methods for producing soluble and active recombinant proteins; new Claims 12 and 13 and original Claim 2 feature methods for producing more stable proteins; new Claim 14 and original Claim 3 feature methods for purifying native bovine alpha-crystallin protein; and new Claim 15 and original Claim 5 feature methods for protecting a protein from proteolysis during purification. As the subject matter of original Claims 1-3 and 5 is similar to the subject matter of new Claims 6-15, the rejections issued in the Office Action mailed November 5, 2002 are applied to corresponding new Claim 6-15 and are addressed accordingly.

Second Supplemental Information Disclosure Statement

A Second Supplemental Information Disclosure Statement (IDS) is being filed concurrently herewith. Entry of the Second Supplemental IDS is respectfully requested.

Objections to the Specification and Claims

The Examiner has made a number of objections to the specification and claims. As noted above, Applicant has amended the specification and claims to overcome the objections. In light of these amendments, Applicant submits that the objections are now moot. Withdrawal of the objections is respectfully requested.

Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 1-5 stand rejected under U.S.C. § 112, second paragraph as being indefinite for failing to point out and distinctly claim the subject matter which Applicant regards as the invention. Applicant again points out that Claims 1-5 have been cancelled herein and new Claims 6-15 have been added. In view of the cancellation of the original claims and addition of new claims, Applicant believe that all rejections under U.S.C. § 112, second paragraph have been overcome. Withdrawal of the rejections is respectfully requested.

Rejections Under 35 U.S.C. § 103(a)

Claims 1-5 stand rejected under 35 U.S.C. §103(a) as being obvious over a number of cited references described in detail below. Analyzing a claimed invention for obviousness requires the sometimes difficult, but critical step of casting the mind back to the time the invention was made, to consider the thinking of one of ordinary skill in the art, guided only by the prior art references and the then-accepted wisdom in the field. Where the claimed invention is rejected as obvious in view of a combination of references, 35 U.S.C. § 103 requires (1) " there must be some suggestion or motivation, ... to modify the reference or combine reference teachings" (2) "there must be a reasonable expectation of success" (3) "the prior art reference (or references when combined) must teach or suggest all claim limitations" and (4) "[t]he teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art" (M.P.E.P. 2142).

Claim 1 stands rejected under 35 U.S.C. § 103(a) as being obvious over Liang et al. (Developmental Biology, 207:445-456, 1999) (hereafter "Liang") taken with Pilon et al. (Biotechnol. Prog., 12:331-337, 1996) (hereafter "Pilon") and Wittliff et al. (Journal of Biological Chemistry, 265:22016-22022, 1990) (hereafter "Wittliff"). Applicant submits that original Claim 1 corresponds to new Claims 6-11. As such, Applicant addresses the rejection as it applies to new Claims 6-11.

Independent Claim 6 recites a method for producing soluble and active recombinant protein comprising expressing an insoluble protein as a fusion protein with an alpha-crystallin type protein or a fragment thereof comprising an active domain. Independent Claim 9 recites a method of increasing the solubility of a first protein, comprising expressing the first protein as fusion protein with a second protein consisting essentially of an alpha-crystallin type protein or a fragment thereof comprising an active domain.

Applicant asserts that Claims 6 and 9 are not obvious over Liang, taken with Pilon and Wittliff. For the reasons set forth below, one of skill in the art, at the time the invention was made, would not be motivated to combine the cited art references. Moreover, even if the motivation for combination existed, there is no reasonable expectation of success to arrive at the claimed invention. The combination of the Liang, Pilon, and Wittliff references do not meet the

criteria to establish a *prima facie* case of obviousness and, thus, do not render the claimed invention as obvious.

Liang discloses that *Artemia* larvae and bacteria that contain the *Artemia franciscana* protein p26 are more thermotolerant than *Artemia* larvae and bacteria that do not contain p26. Liang suggests that p26 protects macromolecules in *Artemia* embryos from irreversible denaturation and that p26 may have chaperone-like activity. Liang does not teach or suggest that p26, or any other alpha-crystallin type protein can be used to produce soluble and active recombinant proteins by expressing a fusion protein of p26 and an insoluble protein in bacteria, as recited in Claim 6. Nor does Liang provide motivation to modify any of its teachings to achieve the method recited in Claims 6 or 9, or a reasonable expectation that the methods as recited in Claims 6 and 9 would be successful.

Pilon discloses that ubiquitin with a 17 amino acid peptide extension can be expressed at high levels in *E. coli*, and that this ubiquitin-peptide fusion can be recovered efficiently.

Wittliff discloses that the human estrogen receptor protein can be expressed as a ubiquitin fusion in *E. coli*. When ubiquitin is cleaved from the fusion protein, the result is an estrogen receptor that can bind its ligands.

Neither Pilon or Wittliff make up for the deficiencies in Liang. Both Pilon and Wittliff disclose that ubiquitin can be used in fusion proteins to express a peptide or a protein in *E. coli*. Neither Pilon or Wittliff suggest a reason why one would want to modify the described methods of generating ubiquitin-peptide or ubiquitin-protein fusions to obtain the methods as recited in Claims 6 or 9. Nor do Pilon or Wittliff teach or suggest that p26 can be used as an alternative to ubiquitin for producing soluble and active recombinant proteins in bacteria.

In light of the above, Applicant submits that Claims 6 and 9 are not obvious in view of the combined teachings of Liang, Pilon, and Wittliff. Furthermore, Claims 7, 8, 10, and 11, depend from either Claim 6 (Claims 7 and 8) or Claim 9 (Claims 10 and 11) and are subject to each limitation of the respective independent claims. As such, Applicant asserts that Claims 7, 8, 10, and 11 are not obvious in view of Liang, Pilon, and Wittliff. Reconsideration and withdrawal of the rejection are respectfully requested.

Claim 2 stands rejected under 35 U.S.C. § 103(a) as being obvious over Wagner et al. (Archives of Biochemistry and Biophysics, 323:455-462, 1995) (hereafter "Wagner") taken with

Adams et al. (Cancer Research, 59:2615-2622, 1999) (hereafter "Adams") and Martens et al. (U.S. Patent No. 5,804,417) (hereafter "Martens"). Applicant submits that original Claim 2 corresponds to new Claims 12-13. As such, Applicant addresses the rejection as it applies to new Claims 12-13.

As stated above, the cited combination of references do not make obvious the claimed invention as there is no motivation to combine the references or reasonable expectation of success if the teachings of the cited references were combined. Claim 12 recites a method of increasing the stability of a first protein, comprising expressing the first protein as a fusion protein with a second protein consisting essentially of an alpha-A-crystallin protein in bacteria; purifying the fusion protein; and removing the alpha-crystallin type protein or fragment thereof from the fusion protein, thereby resulting in the first protein.

Wagner discloses that the alpha-crystallin protein has chaperone activity and that it inhibits multicatalytic proteinase complex-catalyzed hydrolysis of a peptide. Wagner does not teach or suggest methods for increasing the stability of a protein by making a fusion with an alpha-A-crystallin protein as recited in Claims 12 and 13.

Martens discloses the use of the protein 7B2 as a chaperone protein *in vitro* or *in vivo*. The 7B2 protein is expressed as a fusion protein with a glutathione-S-transferase protein (GST), but it appears that the purpose of expressing this fusion protein was to purify 7B2 protein; no indication is provided that the purpose was to increase the stability of GST, and no data is provided to indicate that expression of the 7B2-GST fusion protein resulted in increased stability of GST. Martens does not provide any motivation for using a protein other than 7B2 for any purpose, and in particular, does not suggest increasing protein stability in *in vitro* or *in vivo* methods using any protein. Thus, Martens does not provide motivation to use an alpha-A-crystallin protein to improve protein stability.

Adams discloses the use of a unique series of boronic acid proteosome inhibitors as potential antitumor agents. Adams does not teach or suggest methods for increasing the stability of a protein by making a fusion with an alpha-A-crystallin protein as recited in Claims 12 and 13. Therefore, the teachings of Adams does not compensate for the shortfalls of Wagner and Martens in teaching methods for increasing protein stability as recited in Claims 12 and 13. In light of the above comments, withdrawal of the rejection is respectfully requested.

Claim 3 stands rejected under 35 U.S.C. § 103(a) as being obvious over Gopalakrishnan et al. (Invest. Opthal. Vis. Sci., 33:2936-2941, 1992) (hereafter "Gopalakrishnan") taken with Reddy et al. (Journal of Biological Chemistry, 275:4565-4570, 2000) (hereafter "Reddy") and Wilson et al. (U.S. Patent No. 6,310,186 B1) (hereafter "Wilson"). Applicant submits that original Claim 3 corresponds to new Claim 14. As such, Applicant addresses the rejection as it applies to new Claim 14.

Claim 14 recites a method of purifying native bovine alpha-crystallin protein, comprising the steps of: a) contacting a protein fraction comprising an alpha crystallin protein with a glycine solution having a pH of approximately 2.5; b) size filtering the fraction of step a); c) neutralizing the fraction containing the alpha-crystallin protein; and d) buffering the alpha-crystallin protein to a pH of approximately 8.

Gopalakrishnan discloses a method of purifying native bovine alpha-crystallin. The method involves homogenizing bovine lens cortex in 50 mmol/L Tris Cl, pH 7.4; centrifuging the solution at a speed of 5000 x g; dialyzing the supernatant against a solution containing Na_2SO_4 and $NaHPO_4$; subjecting the dialyzed supernatant to high-performance liquid chromatography gel filtration; and obtaining the fraction corresponding to alpha-crystallin. Gopalakrishnan does not disclose the method as recited in Claim 14, because Gopalakrishnan does not disclose contacting a protein fraction comprising an alpha-crystallin protein with a glycine solution having a pH of approximately 2.5. Nor does Gopalakrishnan suggest that such a solution can be used to purify native bovine alpha-crystallin.

Reddy does not make up for the insufficiencies of Gopalakrishnan. Reddy discloses a method for purifying human alphaA- and alphaB crystallin. The method disclosed by Reddy is that of Andley et al. (Journal of Biological Chemistry, 271:31973-31980, 1996; submitted as reference AY2 in the Second Supplemental Information Disclosure Statement, filed concurrently herewith). Andley discloses a method for purifying alphaA crystallin, and this method does not state that a glycine solution having a pH of approximately 2.5 is used in any part of the purification process. Thus, Reddy does not teach the missing step of Gopalakrishnan, and the combined teachings of Gopalakrishnan and Reddy do not teach all of the elements of Claim 14.

Furthermore, the teachings of Wilson do not compensate for the deficiencies of Gopalakrishnan and Reddy. Wilson discloses a method for separating bacterial endotoxin

associated as a contaminant with biological material. The method involves a hydrophobic solid separation process which does not involve contacting a protein to be purified with a glycine solution have a pH of approximately 2.5. Wilson also discloses that the partially purified product of the hydrophobic separation process can be further purified by using chromatography (Column 4, lines 37-39). In this additional step of purifying by chromatography however, the use of a glycine solution have a pH of approximately 2.5 is not disclosed. Thus Wilson, does not make up for the insufficiencies of Gopalakrishnan or Reddy; and the combined teaching of Gopalakrishnan, Reddy, and Wilson do not teach all of the elements of Claim 14.

In light of the above comments, Applicant submits that Claim 14 is not obvious over Gopalakrishnan taken with Reddy and Wilson. Withdrawal of the rejection is respectfully requested.

Claim 4 stands rejected under 35 U.S.C. § 103(a) as being obvious over Cobb et al. (Journal of Biological Chemistry, 275:6664-6672, 2000) taken with Guo et al. (Biochemical and Biophysical Research Communications, 270:183-189, 2000).

Applicant points out that Claim 4 has been cancelled herein, thus rendering the rejection moot. Withdrawal of the rejection is respectfully requested.

Claim 5 stands rejected under 35 U.S.C. § 103(a) as being obvious over Gopalakrishnan taken with Wagner et al. Applicant submits that original Claim 5 corresponds to new Claim 15. As such, Applicant addresses the rejection as it applies to new Claim 15.

Claim 15 recites a method for protecting a protein sample from proteolysis during purification, comprising applying a sample comprising the protein to a chromatographic pre-column filter comprising bovine alpha-crystallin protein coupled to a chromatography resin.

Applicant submits that the combined teachings of Gopalakrishnan and Wagner do not render the method as recited in Claim 15 obvious. Gopalakrishnan discloses a method of covalently coupling bovine alpha-crystallin to Sepharose and using it to study changes in the subunit exchange of alpha-crystallin present in high molecular weight versus low molecular weight aggregates of the human lens. The method involves crosslinking bovine alpha-crystallin to cyanogen-bromide Sepharose, and loading the Sepharose-alpha-crystallin complex onto a column. The alpha-crystallin subunit exchange studies were then carried out on the column. No other columns or pre-column filters were used in the methods of Gopalakrishnan. Therefore,

Gopalakrishnan does not teach a method for protecting a protein from proteolysis during purification by applying a sample comprising the protein to a chromatographic pre-column filter, comprising bovine alpha-crystallin protein coupled to a chromatography resin as recited in Claim 15.

Wagner discloses that the alpha-crystallin has protein chaperone activity and that it inhibits multicatalytic proteinase complex-catalyzed hydrolysis of a peptide. Wagner does not teach or suggest that bovine alpha-crystallin can be coupled to a chromatography resin, applied to a pre-column filter, and used to protect a protein from proteolysis. Thus, Wagner does not teach or suggest the method as recited in Claim 15.

In view of the above comments, reconsideration and withdrawal of the rejection are respectfully requested.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (978) 341-0036.

Respectfully submitted,

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Dated: April 7, 2003

MARKED UP VERSION OF AMENDMENTS

Specification Amendments Under 37 C.F.R. § 1.121(b)(1)(iii)

Replace the paragraph at page 3, lines 12 through 16 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

In another aspect of the present invention, a device that acts as a pre-column filter for reducing unwanted proteolysis on a chromatography column during purification of a target protein is provided. The pre-column filter is an affinity chromatography resin useful for removing proteases from crude protein extracts. In one embodiment, the pre-column filter comprises bovine lens alpha crystallin coupled to cyanogen bromide activated sepharose (CNBr-sepharose).

Replace the paragraph at page 4, line 16 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

FIG. 3 is a digital image of [an] a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE gel) showing purified BC-pepsinogen.

Replace the paragraph at page 4, lines 18-23 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

FIG. 4 shows a digital image of a 12% SDS PAGE gel of p26 protein purified by nickel affinity chromatography resin. Because p26 is a multi-oligomer, it has a tendency to elute over several fractions, even when a sharp gradient is provided. Fractions identified using the SDS gel and containing p26 are dialyzed into Pipes magnesium buffer (20 mM piperazine-1,4-bis(2-

ethanesulphonic acid (Pipes) pH 7.0, 1 mM $MgCl_2$). Following dialysis the target protein was stored at -20°C and used in less than 1 week for kinetic assays and chromatography experiments.

Replace the paragraph at page 5, lines 13-26 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

Referring to FIG. 1, an expression vector consisting of a gene fusion between an unstable or insoluble protein could be stabilized or protected from proteolysis with the appropriate class of small molecular chaperone/alpha crystallin type proteins such as p26 from Artemia, SicA from Salmonella and alpha-A-crystallin protein from bovine lens. Unexpectedly it was shown that the protein p26 from *Artemia* has an active domain that can assist in the formation of soluble proteins based on its properties as an alpha-crystallin type protein. It was determined that full-length p26 protein is completely insoluble when express in *E. coli* at [37-C] 37°C. It is expected that proteins with a similar structural fold such as the SicA protein from *Salmonella typhimurium* could be substituted for p26. These results indicate that p26 and SicA are functionally very similar. It is also envisioned that the chaperone could be co-expressed on two different promoters either on the same plasmid or on different plasmids in the bacteria. The advantages of co-expression are that it would not require the removal of a fusion tag (such as thrombin) prior to purifying the protein of interest.

Replace the paragraph at page 9, line 15 through page 10, line 10 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

The entire p26 protein was expressed by growing *E. coli* to an optical density of about 1.0 and then inducing 4 hours with 1 mM IPTG. P26 protein was purified with Ni^{2+}-NTA sepharose (Qiagen, cat #30410,) using the detergent DECAMEG (Calbiochem, cat# 373272, lot# b27260) to gently strip the protein contaminants that are nonspecifically bound to the p26 in the crude extract. At 37°C, all of the protein is in inclusion bodies, thus, an inclusion body prep was used to purify the protein. Briefly, following lysis by 3 x 15 sec bursts of sonication, the cell extract is centrifuged at 13 k revolutions per minute (RPM) for 15 minutes. The pellet was resuspended in low buffer (10